Pest Management Grants Final Report

Contract No. 98-0277

Use of a Natural Product to Stimulate Sclerotial Germination of *Sclerotium cepivorum* for the Control White Rot of Onions and Garlic

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The statements and conclusions in this report are those of the contractor and not necessarily those of the California Department of Pesticide Regulation. The mention of commercial products, their source, or their use in connection with materials reported herein is not to be construed as actual or implied endorsement of such products.

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Abstract

White rot of onion and garlic, caused by the soilborne fungus *Sclerotium cepivorum*, is a worldwide threat to *Allium* production. No economical control measures currently exist and once a field is infested, it will remain so indefinitely since sclerotia of the fungus remain dormant in the absence of *Allium* plants. Hence, infested fields are often forever abandoned from further onion or garlic production. Sclerotia germinate only in response to root exudation of specific volatile sulfides and thiols. If these sulfides can be applied to the ground in the absence of an *Allium* crop, the sclerotia may be "tricked" into germinating. In the absence of a host, the mycelium from germinating sclerotia persist for a while, then die after exhausting nutrient reserves. One material that may act as a sclerotial germinating stimulant is garlic powder used in the food processing industry. Being a natural plant product, garlic powder is a readily available, environmentally compatible, renewable resource that should pose no problem to worker safety. In this study garlic powder was incorporated into the soil at three rates. Periodically thereafter, the number of viable sclerotia in the soil dropped significantly in the treated plots, approaching the degree of sclerotia mortality (90% in some treatments) achieved with an application of methyl bromide. However, sclerotia mortality from the garlic powder treatment was apparently limited to the upper 6 inches of the soil profile because once the garlic roots of a crop planted the next season grew to that depth, the plants became infected. At the season's end, the incidence of white rot was high in all treatments except

in the plots treated with methyl bromide. It is hypothesized that the effects of the garlic powder are limited to the zone of application. Thus, a thorough incorporation of garlic powder throughout the root zone is necessary to achieve the desired level of control.

Executive Summary

White rot of onion and garlic, caused by the soilborne fungus *Sclerotium cepivorum*, is a worldwide threat to *Allium* production. No economical control measures currently exist and once a field is infested, it will remain so indefinitely since sclerotia of the fungus remain dormant in the absence of *Allium* plants. Hence, infested fields are often forever abandoned from further onion or garlic production. Sclerotia germinate only in response to root exudation of specific volatile sulfides and thiols. If these sulfides can be applied to the ground in the absence of an *Allium* crop, the sclerotia may be "tricked" into germinating. In the absence of a host, the mycelium from germinating sclerotia persist for a while, then die after exhausting nutrient reserves. In this study, garlic powder and synthetic garlic oil were incorporated into the soil in two trials. Periodically thereafter, the number of viable sclerotia in the soil dropped significantly in the treated plots, approaching the degree of sclerotia mortality achieved with an application of methyl bromide. Garlic was planted in the plots 8 to 10 months after treatment. Despite significant declines in sclerotia populations in the plots amended with garlic powder and garlic oil, disease incidence was high and yields were low. Failure to incorporate the test materials deeper than about 6 inches (less than that explored by the roots of the subsequent garlic crop) may have been a limiting factor in the success of the treatments. It is hypothesized that the effects of the garlic powder are limited to the zone of application. Thus, a thorough incorporation of garlic powder throughout the root zone is necessary to achieve the desired level of control. Therefore, we have initiated another trial which will better incorporate the garlic powder and oil. That trial is in progress.

Body of Report

Introduction. White rot of onion and garlic, caused by the soilborne fungus *Sclerotium cepivorum* Berk, is a worldwide threat to *Allium* production. The disease is extremely serious on these crops - an inoculum density of a single sclerotium in a liter of field soil can potentially result in crop failure and no economical control measures currently exist. Furthermore, once a field is infested, it will remain so for at least 40 years and probably longer since sclerotia of the fungus remain dormant indefinitely in the absence of *Allium* plants. Loss estimates to this disease are difficult to ascertain because once identified in a field, growers are forced to grow other, nonsusceptible (non-*Allium*) crops. Hence, infested fields are often forever abandoned from further onion or garlic production.

The white rot fungus produces no functional spores. Instead, it propagates only by the production of round, poppy seed-sized sclerotia produced on the roots of decayed host plants. Sclerotia spread in mass movement of soil or water, on animals (at least theoretically), and especially on infested plant parts. Once introduced into an area, *S. cepivorum* is gradually spread on contaminated equipment or planting materials, and slowly the production of garlic and onions in the entire region is threatened. Garlic culture is perhaps the principal mode of movement since it is propagated vegetatively, and garlic bulbs and cloves are sufficiently large that an infestation might go unnoticed. In any case, the disease is spreading throughout western North America.

Traditional methods to control white rot are either economically prohibitive or ineffective. Currently, the only effective method of control is tarped fumigation with methyl bromide. This method may kill 99% or more sclerotia in the soil, but does not result in complete eradication. Therefore, retreatment has to made on an ongoing basis since very few viable sclerotia remaining in a field can result in disastrous consequences in *Allium* production. Because retreatment with methyl bromide may be necessary, its use may not be cost effective. Moreover, the material itself is scheduled to be phased out for use in a few years according to the U.S. Environmental Protection Agency, which initiated action under the Clean Air Act for a phase out of chemicals threatening the ozone layer by the year 2005.

White rot is a disease limited to *Allium* crops. The fungus successfully colonizes only *Allium* plants and sclerotia germinate only in response to exudation by *Allium* roots. These exudates contain alkyl and alkenyl-L-cysteine sulphoxides, which are metabolized by the soil microflora to yield a range of volatile thiols and sulfides that activate the dormant sclerotia. The specific reaction between sclerotia and sulphoxides or their breakdown products suggests a possible use of these sclerotial germination stimulants for controlling white rot disease. If these thiols can be applied to the ground in the absence of an *Allium* crop, the sclerotia may be "tricked" into germinating. In the absence of a host, the mycelium from germinating sclerotia persist for periods ranging from a few days to several weeks depending on the soil temperature, then die after exhausting nutrient reserves.

One natural sclerotial stimulant from *Allium* spp. is diallyl disulfide, which is also recoverable from the distillation of petroleum. Recent research demonstrated that diallyl disulfide distributed through the soil profile in the absence of an *Allium* crop in an infested field forced 90-99% of the sclerotia to germinate. This degree of germination resulted in disease control that is similar to control achieved with methyl bromide fumigation

Garlic powder, a deregulated product from dehydrated garlic bulbs used in food processing, is another sclerotial stimulant. In last year's tests, garlic powder effectively reduced sclerotia viability in the upper 5 or 6 inches of the soil profile. However, based on the high incidence of disease in a subsequent garlic crop, garlic powder failed to reduce sclerotia populations below that depth. This year, we have initiated trials to incorporate garlic powder at a greater depth.

Materials and Methods. The first experiments were conducted in two grower fields, one in Kern County and one in San Benito County. The experimental design was a randomized complete block design with four replications per treatment. Experimental units were 20X20 ft. Data was collected from the center 10X10 ft. Plots were separated by a 20 ft. border to prevent cross contamination of the volatile compounds. Treatments included a nontreated control, garlic powder applied at 50, 100, or 200 lbs/acre, diallyl disulfide at 5 mls/m^2 (5.3 gal/acre), an effluent product from a garlic processing plant, and methyl bromide (98% methyl bromide, 2% chloropicrin) at 400 pounds per acre. The garlic powder was mixed into sand and applied in a hand-held fertilizer applicator. Methyl bromide was applied under tarp by a commercial applicator. All treatments were applied February 26, 1998 in Kern County and the garlic powder treatments were applied April 3, 1998 in San Benito County.

Soil populations of sclerotia were determined at the time of soil treatment and at approximately two-month intervals thereafter. On each sampling date, a composited sample of ten, 1 cm-diameter core subsamples per plot were collected from the soil surface to 6 inches deep. From each sample, 500 cc of soil was directly assayed (or air-dried at room temperature prior to assay) by wet soil sieving. After sieving, soil residue was frozen until observation and viability testing. Sclerotia were identified and plated on water agar amended with 25 ppm streptomycin to observe the proportion from which the fungus grew following disinfestation with mild bleach (0.05% NaOCl) to rid the surface of sclerotia of superficial contaminants. A garlic crop was planted in December following treatment at both locations to evaluate the disease control. In the spring, plants were gently lifted to examine root systems. White rot severity was evaluated on a scale of 0-6, where 0=0%, 1=7%, 2=21%, 3=50%, 4=79%, 5=93%, and 6=100% of the root system decayed. The percentages of decayed root systems of about 20 individual plants from each plot were recorded.

A new trial in Kern County has been initiated. Treatments (as main plots) include garlic powder at 100 lbs/acre (split into two applications, one applied in November, 1999, and one to be made in April, 2000), synthetic garlic oil at 5.3 gal/acre (also a split application), methyl bromide at 400 lbs/acre, and a nontreated control. Subplots will include the fungicide Folicur (applied in the planting furrow at time of planting), and a nontreated control. Plots are 50 X 100 ft. Garlic powder and the garlic oil were applied to the surface of the soil, plowed, then mulched for optimum incorporation.

Results: Populations of sclerotia in soil at both field sites were high. In Kern County, all treatments significantly reduced sclerotia numbers beginning 2 months after treatment (Fig. 1). Sclerotia viability was most effectively reduced by 100 or 200 pounds of garlic powder, diallyl disulfide (DADS), and the methyl bromide. There were no significant differences among these treatments. In the San Benito County trial, results were similar (Fig. 2). The two high rates of garlic powder were generally more effective in reducing the number of sclerotia in the soil than the low rate.

In the Kern County experiment, neither control of white rot nor garlic yields were improved by soil amendment with garlic powder (Tables 1 and 2). Methyl bromide, however, reduced the severity of disease and resulted in increased yields. In the San Benito experiment, all rates of garlic powder slightly but significantly reduce white rot severity. The high rate of garlic powder resulted in increased bulb weight and all rates of garlic powder significantly increased plant top weight.

Discussion: When applied at 100 or 200 pounds per acre, garlic powder reduced the inoculum density of soilborne sclerotia of *Sclerotia cepivorum* as effectively as methyl bromide, currently the only effective method of control (results presented in the previous report). However, sclerotia below the depth of garlic powder incorporation survived and caused significant disease and yield loss. For this reason, another trial was initiated that will better incorporate the garlic powder throughout the root zone. Results from the initial trials are encouraging, however, since the degree of sclerotia mortality approached 100% in the treated zone. Furthermore, white rot severity in the subsequent garlic crop was significantly reduced by the application of garlic powder (although levels of disease remained substantial). Hence, garlic powder appears to be an effective alternative to methyl bromide for white rot control if optimum application methodology can be determined.

Summary and Conclusions: In this study, germination stimulants of sclerotia of *Sclerotium cepivorum*, including garlic powder, diallyl disulfide, and an effluent from a garlic processing plant, were incorporated into the soil in replicated plots in two commercial fields naturally infested with the fungus.

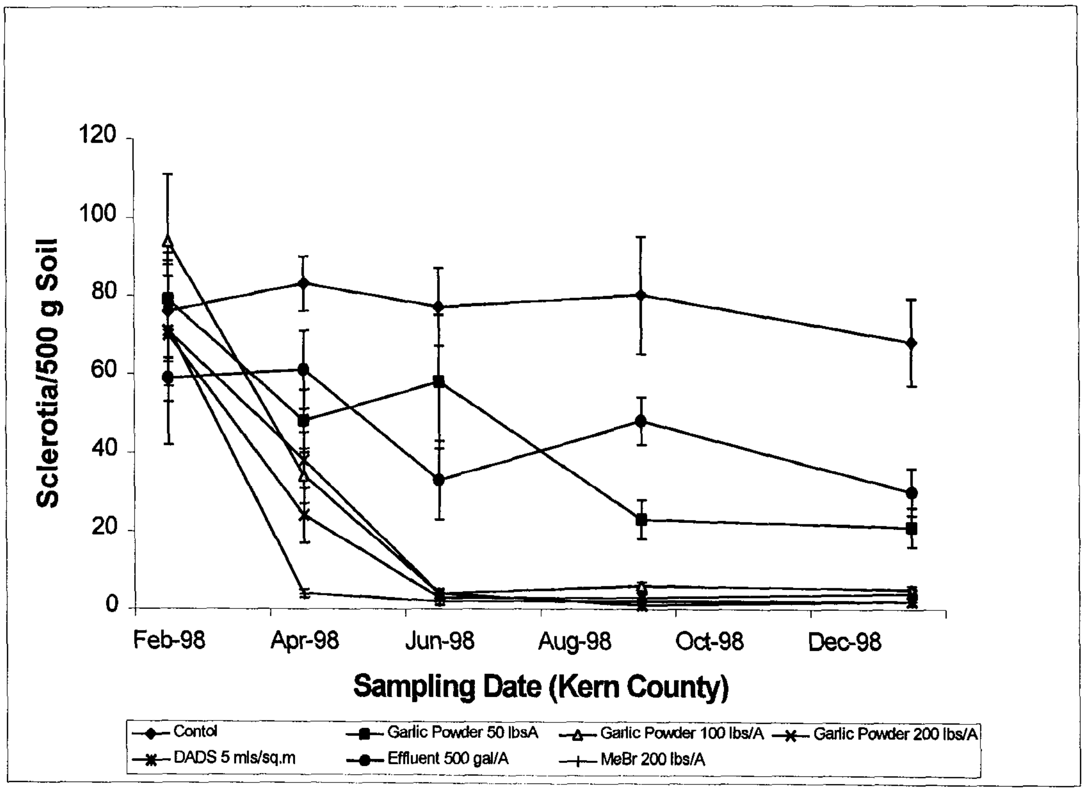
The number of viable sclerotia in the soil treated with garlic powder was significantly reduced. At the high rates (100 or 200 pounds of garlic powder per acre), the degree of sclerotia mortality achieved approached that achieved with an application of methyl bromide. Diallyl disulfide was equally effective. The effluent reduced sclerotia numbers, but not to the degree of the other treatments.

In the Kern County experiment, neither control of white rot nor garlic yields were improved by soil amendment with garlic powder despite the reduction of sclerotia. Methyl bromide, however, reduced the severity of disease and resulted in increased yields. In the San Benito experiment, all rates of garlic powder slightly but significantly reduce white rot severity. The high rate of garlic powder resulted in increased bulb weight and all rates of garlic powder significantly increased plant top weight.

Apparently, sclerotia below the depth of garlic powder incorporation survived and caused significant disease and yield loss as the roots grew below the treated zone. Better incorporation of the sclerotial stimulants down through the soil profile may result in maximum mortality of sclerotia, and therefore disease control.

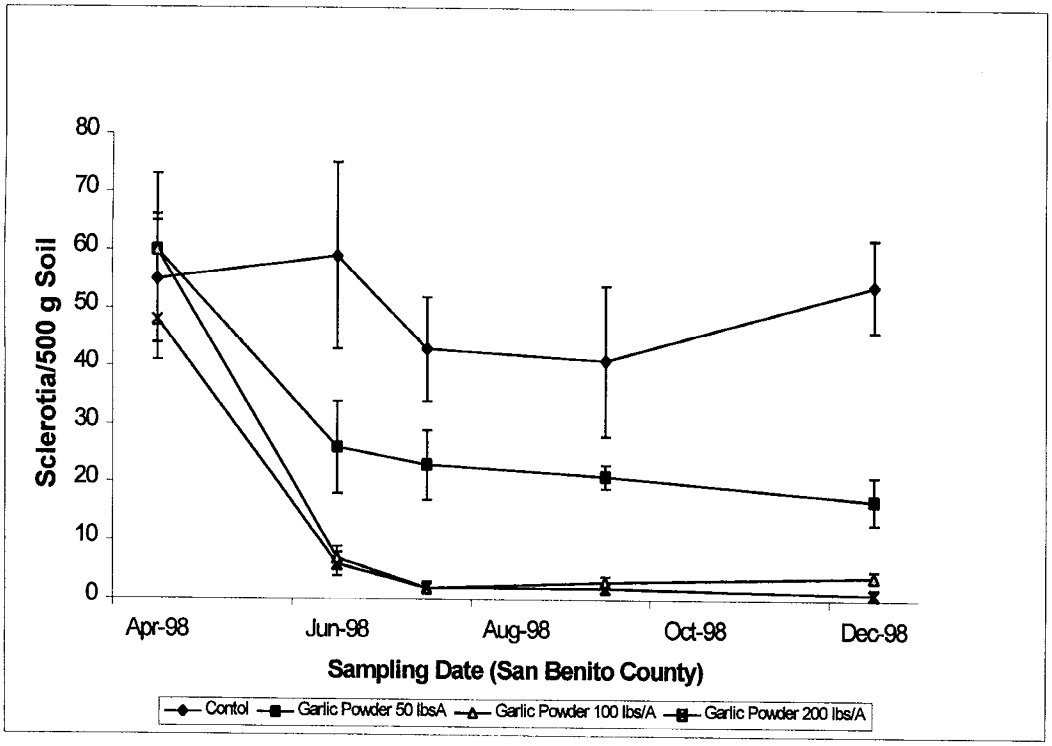
Appendix

Figure 1. Effect of sclerotia stimulants on numbers of viable sclerotia of *Sclerotium cepivorum*, Kern County



The vertical bars indicate the standard errors of the means.

Figure 2. Effect of sclerotia stimulants on numbers of viable sclerotia of *Sclerotium cepivorum*, San Benito County



The vertical bars indicate the standard errors of the means.

Table 1. Severity of white rot following soil treatment with sclerotial stimulants

	Kern County White rot severity(%)[z]	San Benito County White rot severity(%)[z]
None	94.0 a	88.4 a
Garlic powder 50 lbs/acre	92.2 a	77.3 b
Garlic powder 100 lbs/acre	97.7 a	71.0 b
Garlic powder 200 lbs/acre	95.3 a	72.5 b
Diallyl disulfide 5 mls/m^2	92.9 a	-
Effluent	96.6 a	-
Methyl bromide 400 lbs/acre	55.0 b	-

[z] White rot severity was evaluated on a scale of 0-6, where 0=0%, 1=7%, 2=21%, 3=50%, 4=79%, 5=93%, and 6=100% of the root system decayed. Percentages of decayed root systems of individual plants (means of about 20 plants per plot) are presented.

Table 2. Garlic yield following soil treatment with sclerotial stimulants

Treatment	Kern County Bulb dry wt (g)	Kern County Top dry wt (g)	San Benito County Bulb dry wt (g)	San Benito County Top dry wt (g)
None	30.4 a	5.6 a	29.5 a	40.0 c
Garlic powder 50 lbs/acre	23.8 a	7.5 a	38.2 a	66.5 b
Garlic powder 100 lbs/acre	26.1 a	4.3 a	41.3 a	65.6 b
Garlic powder 200 lbs/acre	30.9 a	4.5 a	59.9 b	76.5 a
Diallyl disulfide 5 mls/m^2	22.7 a	11.0 a	-	-
Effluent	29.1 a	5.0 a	-	-
Methyl bromide 400 lbs/acre	56.3 b	51.4 b	-	-